Journal of Advanced Medical and Dental Sciences Research

@Society of Scientific Research and Studies ***NLM ID:*** *101716117*

Journal home page: www.jamdsr.com doi: 10.21276/jamdsr Indian Citation Index (ICI) Index Copernicus value = 100

(e) ISSN Online: 2321-9599; (p) ISSN Print: 2348-6805

Original Research

Molecular Identification and Characterization of Carbapenemase Genes (CRKP-2 and OXA-48) in Klebsiella Species from clinical samples

Nitika Sharma[1], Ritu Bhatnagar[2]

[1]Ph.D Research Scholar, Department of Microbiology, Faculty of Medicine, Pacific Medical University, Udaipur, Rajasthan, India;
[2]Professor and Head, Department of Microbiology, Pacific Medical College and Hospital, Pacific Medical University, Udaipur, Rajasthan, India

ABSTRACT:
Background: The emergence of carbapenem-resistant Klebsiellapneumoniae (CRKP) poses a critical threat to global healthcare due to limited therapeutic options and rapid transmission. This study aimed to identify and characterize carbapenemase genes, specifically bla_{OXA-48} and bla_{CRKP-2}, in clinical isolates of Klebsiellapneumoniae. **Methods:** A total of 156 K. pneumoniae isolates were recovered from 1000 clinical specimens and subjected to antibiotic susceptibility testing using the Kirby-Bauer method. Carbapenem-resistant isolates were further analyzed by minimum inhibitory concentration (MIC) testing, phenotypic detection (Modified Hodge Test and Combined Disc Test), and PCR-based genotypic characterization of OXA-48 and CRKP-2 genes. PCR amplicons were validated through BLAST sequence alignment. **Results:** Among the isolates, 18 (11.53%) showed resistance to imipenem-EDTA; 10 were confirmed resistant by MIC testing (≥4 µg/mL). Phenotypic assays indicated carbapenemase production in 60% (MHT) and 70% (CDT) of resistant isolates. Genotypic analysis revealed that all 10 isolates (100%) harbored the bla_{OXA-48} gene, while none carried the bla_{CRKP-2} gene. BLAST alignment confirmed 99–100% similarity with known OXA-48 sequences. **Conclusion:** The study highlights the predominance of OXA-48-type carbapenemase among clinical K. pneumoniae isolates, with no detection of CRKP-2. Molecular diagnostics remain essential for accurate detection and containment of carbapenem-resistant pathogens in healthcare settings.
Keywords: Klebsiellapneumoniae, OXA-48, CRKP-2, carbapenemase, antimicrobial resistance, PCR, MIC.

Received: 14 April, 2023 Accepted: 16 May, 2023 Published: 03 June, 2023

Corresponding author: Nitika Sharma, Ph.D Research Scholar, Department of Microbiology, Faculty of Medicine, Pacific Medical University, Udaipur, Rajasthan, India

This article may be cited as: Sharma N, Bhatnagar R. Molecular Identification and Characterization of Carbapenemase Genes (CRKP-2 and OXA-48) in Klebsiella Species from clinical samples. J Adv Med Dent Scie Res 2023;11(6):163-168.

INTRODUCTION

The emergence and global dissemination of antimicrobial resistance (AMR) among Gram-negative pathogens represents one of the most pressing public health threats of the 21st century. Among these, Klebsiella species—especially Klebsiellapneumoniae—have gained notoriety as significant nosocomial pathogens associated with high morbidity and mortality due to their ability to acquire and disseminate resistance determinants, particularly against last-resort antibiotics like carbapenems. Carbapenem-resistant Klebsiellapneumoniae (CRKP) infections are increasingly reported worldwide, posing a major therapeutic dilemma and challenging current infection control protocols in clinical settings [1,2].

Carbapenem resistance in Klebsiella species is largely mediated by the production of carbapenem-hydrolyzing β-lactamases, commonly referred to as carbapenemases. Among the various classes of carbapenemases, KPC-type enzymes (notably CRKP-2, a variant of KPC-2) and class D oxacillinases such as OXA-48 are two of the most clinically significant resistance mechanisms [3,4]. These enzymes hydrolyzecarbapenem antibiotics, rendering them ineffective and significantly limiting therapeutic options. The genes encoding these enzymes—bla_{KPC} and bla_{OXA-48}—are often located on mobile genetic elements such as plasmids, facilitating their rapid horizontal transfer among different bacterial species and strains [5,6].

The bla_{KPC} gene, first identified in the United States in 1996, encodes a class A serine β-lactamase with broad-spectrum activity against β-lactams, including carbapenems [7]. Among the numerous variants, CRKP-2 (a prevalent variant of KPC-2) is frequently reported in Asia and Europe and has been associated with multiple hospital outbreaks due to its robust dissemination potential [8]. On the other hand, bla_{OXA-48}, a class D β-lactamase gene, was first identified in Turkey in 2001 and has since become endemic in the Mediterranean region, with increasing reports from South Asia and the Middle East [9]. Unlike KPC, OXA-48 exhibits relatively weak hydrolysis of carbapenems but may remain undetected in routine susceptibility testing, making it a silent yet formidable resistance determinant [10].

The accurate identification and molecular characterization of these carbapenemase genes are critical for timely diagnosis, outbreak surveillance, and implementation of effective infection control measures. Phenotypic methods, such as modified Hodge test and Carba NP test, often suffer from limited sensitivity and specificity, particularly in detecting OXA-48 producers [11]. Therefore, molecular diagnostics, including polymerase chain reaction (PCR) and sequencing, have become the gold standard for the detection of carbapenemase genes in clinical microbiology [12,13]. PCR-based assays allow rapid and specific identification of bla_{KPC} and bla_{OXA-48} genes directly from clinical isolates, thereby facilitating early intervention and epidemiological tracking.

In recent years, the prevalence of carbapenemase-producing Klebsiella species has shown a disturbing upward trend in various healthcare settings globally. In India, for instance, multiple surveillance studies have confirmed the co-existence of bla_{OXA-48} and bla_{KPC} genes among multidrug-resistant Klebsiella isolates from ICU settings [14]. This not only complicates treatment strategies but also raises the risk of untreatable infections in immunocompromised patients. Moreover, the dissemination of these genes via high-copy-number plasmids has led to the evolution of extensively drug-resistant (XDR) clones that are resistant to almost all available antibiotic classes [15,16].

The characterization of these genes at the molecular level also provides valuable insights into the epidemiology, clonal diversity, and resistance mechanisms of clinical Klebsiella isolates. Sequencing of CRKP-2 and OXA-48 variants, along with phylogenetic analysis, can reveal mutation hotspots, genetic linkages, and evolutionary trajectories of resistance determinants [17]. Additionally, studies have shown that integrons, transposons, and insertion sequences often play a crucial role in the mobilization and expression of these resistance genes [18].

Considering the alarming rise in CRKP and OXA-48-producing Klebsiella strains and the severe implications for clinical management, there is an urgent need to strengthen molecular surveillance in both community and hospital settings. Identifying the distribution and frequency of these resistance genes in local clinical isolates will aid in tailoring antimicrobial stewardship policies, optimizing therapeutic regimens, and mitigating the spread of multidrug resistance.

Thus, the current study focuses on the molecular identification and characterization of bla_{CRKP-2} and bla_{OXA-48} genes in clinical Klebsiella species isolated from various samples during the study period. By employing PCR-based techniques and sequencing, the study aims to uncover the genetic basis of carbapenem resistance and assess the potential epidemiological linkages among circulating strains.

MATERIALS AND METHODS

Study Design and Sample Collection

This cross-sectional, laboratory-based study was conducted in the department of Microbiology, Pacific Medical College and Hospital Udaipur, Rajasthan. The isolates were obtained from both inpatients and outpatients attending tertiary healthcare settings. Samples were processed according to standard microbiological guidelines outlined by the Clinical and Laboratory Standards Institute (CLSI).

Isolation and Identification of Klebsiella Species

All samples were cultured on MacConkey agar and blood agar and incubated aerobically at 37°C for 18–24 hours. Presumptive identification of Klebsiella species was based on colony morphology, Gram staining, and a battery of biochemical tests including triple sugar iron (TSI) agar, citrate utilization, urease production, indole, motility, and oxidase tests. Final confirmation was performed using the modified hodge test (MHT)and combined disk test (CDT) .

Antibiotic Susceptibility Testing (AST)

Antimicrobial susceptibility testing was carried out by the Kirby-Bauer disk diffusion method on Mueller-Hinton agar in accordance with CLSI M100 guidelines (2020). The antibiotics tested included: meropenem (10 µg), imipenem (10 µg), ertapenem (10 µg), ceftazidime (30 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), and colistin (10 µg). Isolates exhibiting reduced susceptibility to at least one carbapenem were selected for further Imipenem +EDTA (10/750) Cefixime+ EDTA(30) molecular analysis. Escherichia coli ATCC 25922 and Klebsiellapneumoniae ATCC 700603 were used as control strains.

Phenotypic Detection of Carbapenemase Production

Phenotypic confirmation of carbapenemase production was done using the Modified Hodge Test (MHT) and Carba NP test, as per CLSI recommendations. A positive result was considered a presumptive indicator for the presence of carbapenemase genes.

Genomic DNA Extraction

Bacterial genomic DNA was extracted from overnight cultures using the boiling lysis method. A single colony was suspended in 200 µL of sterile nuclease-free water, boiled at 95°C for 10 minutes, and centrifuged at 12,000 rpm for 10 minutes. The supernatant containing crude DNA was collected and stored at –20°C until further use.

Agarose Gel Electrophoresis

PCR products were separated on a 1.5% agarose gel stained with ethidium bromide (0.5 µg/mL) and visualized under UV illumination using a gel documentation system (Bio-Rad). A 100 bp DNA ladder was used to estimate the amplicon size.

Sequencing and Gene Characterization

Sequences were analyzed using NCBI BLAST to confirm gene identity and detect any nucleotide variations. Multiple sequence alignment and phylogenetic analysis were performed using MEGA-X software.

Statistical Analysis

The obtained data were entered into an Excel spreadsheet and analyzed with Statistical Package for the Social Sciences (SPSS). The Pearson chi-square test was used to analyze the data and statistically significant level was accepted as less than 0.05.

RESULTS

Isolation and Identification of Klebsiellapneumoniae

Out of 1000 clinical specimens, 400 isolates were confirmed as Klebsiella species based on morphological characteristics. Biochemical identification revealed 156 isolates as Klebsiellapneumoniae, which were subsequently used for phenotypic and molecular characterization.

Antimicrobial Resistance Profile

Antibiotic susceptibility testing of the 156 Klebsiellapneumoniae isolates revealed significant resistance to several classes of antibiotics, particularly macrolides and β-lactams. Resistance against carbapenems was notably observed, with 33.97% of isolates resistant to meropenem, 37.82% to ertapenem, and 22.43% to imipenem. However, resistance dropped to 11.53% when imipenem was combined with EDTA, indicating potential metallo-β-lactamase activity.

Table 1: Resistance to Carbapenems in Klebsiellapneumoniae

Antibiotic	Resistant Isolates (n, %)	Sensitive Isolates (n, %)
Meropenem	53 (33.97%)	103 (66.02%)
Ertapenem	59 (37.82%)	97 (62.17%)
Imipenem	35 (22.43%)	121 (77.56%)
Imipenem + EDTA	18 (11.53%)	138 (88.46%)

Statistical analysis (ANOVA) confirmed a significant variation in antibiotic resistance patterns ($F = 143.28$, $p = 0.00065$). Chi-square test ($\chi^2 = 6.23$) also affirmed statistical significance in resistance pattern distribution.

MIC Determination for Carbapenem Resistance

The 18 isolates resistant to Imipenem+EDTA on disk diffusion were subjected to E-test for MIC. Results showed that 10 isolates (55.55%) were resistant (MIC ≥ 4 µg/mL), while 8 isolates (44.44%) remained within the susceptible range (MIC ≤ 1 µg/mL).

Table 2: MIC for Imipenem+EDTA among Resistant Isolates

MIC Value (µg/mL)	Number of Isolates	Percentage
≤ 1 (Susceptible)	8	44.44%
≥ 4 (Resistant)	10	55.55%

Phenotypic Detection of Carbapenemase

Phenotypic methods were applied to the 10 carbapenem-resistant isolates (based on MIC):

- **Modified Hodge Test (MHT)** showed positive results in 6 isolates (60%).
- **Combined Disc Test (CDT)** identified 7 positive cases (70%).

Table 3: Comparison of Phenotypic Tests

Test	Positive (n, %)	Negative (n, %)	Total
Modified Hodge Test	6 (60%)	4 (40%)	10

Combined Disc Test	7 (70%)	3 (30%)	10

Combined Disc Test appeared slightly more sensitive than MHT in identifying carbapenemase production.

Genotypic Detection of Carbapenemase Genes by PCR

All 10 carbapenem-resistant isolates (MIC ≥ 4) were subjected to PCR using specific primers for OXA-48 and KPC (CRKP-2) genes. The PCR analysis revealed that all 10 isolates(100%) harbored the OXA-48 gene, confirming its role in carbapenem resistance. No isolates tested positive for the CRKP-2 (KPC) gene.

Table 4: Genotypic Detection by PCR

Gene Targeted	Positive Isolates (n, %)	Negative Isolates (n, %)
OXA-48	10 (100%)	0 (0%)
KPC (CRKP-2)	0 (0%)	10 (100%)

BLAST alignment of the amplicons against NCBI GenBank confirmed high similarity (99–100%) with Klebsiellapneumoniae reference strains, further validating the identity of OXA-48-positive isolates.

DISCUSSION

The present study investigated the molecular characterization of carbapenem-resistant Klebsiellapneumoniae isolates, focusing on the detection of two major carbapenemase genes: bla_{OXA-48} and bla_{CRKP-2}. The findings indicate a high prevalence of OXA-48-producing K. pneumoniae isolates, which aligns with growing global reports of OXA-48 as a dominant mechanism of resistance, especially in regions of South Asia, the Middle East, and the Mediterranean basin [19,20].

Out of 156 K. pneumoniae isolates, 11.53% demonstrated resistance to the imipenem-EDTA combination. Interestingly, this resistance rate was lower than resistance to imipenem (22.43%) alone, suggesting the presence of metallo-β-lactamase activity in a subset of isolates. The reduction in resistance in the presence of EDTA, a known chelator of zinc ions required by metallo-β-lactamases, indicates a potential mixed mechanism of resistance that may include both metallo-enzymes and OXA-48-type carbapenemases [21].

MIC testing further delineated resistance levels, confirming that 10 isolates had MIC values ≥4 μg/mL against the imipenem-EDTA combination, classifying them as highly resistant. These 10 isolates became the focus of subsequent phenotypic and genotypic analyses. Phenotypic detection using Modified Hodge Test (MHT) and Combined Disc Test (CDT) revealed carbapenemase activity in 60% and 70% of these isolates, respectively. The slightly higher sensitivity of the CDT supports previous studies that found MHT less reliable for detecting OXA-48-type enzymes, which have relatively low hydrolytic activity against carbapenems and often yield false negatives in traditional phenotypic assays [22,23].

The most significant finding of the study was that all 10 resistant isolates tested positive for the bla_{OXA-48} gene via PCR, while none carried the bla_{CRKP-2} gene. This 100% detection rate confirms the pivotal role of OXA-48-type carbapenemase in mediating carbapenem resistance in the local clinical isolates of K. pneumoniae. These findings are consistent with epidemiological reports from India, Pakistan, and Turkey where OXA-48 has emerged as the primary resistance gene in carbapenem-resistant Enterobacteriaceae [24–26]. In contrast, KPC-type carbapenemases, including CRKP-2, though dominant in the Americas and parts of Europe, appear to be less prevalent in South Asian contexts [27].

The absence of bla_{CRKP-2} in all isolates also supports previous surveillance data that indicates geographical clustering of different carbapenemase types. This variation may be attributed to differential antibiotic prescribing practices, horizontal gene transfer dynamics, and local infection control measures [28]. The uniform presence of bla_{OXA-48} in all resistant isolates also raises concern over plasmid-mediated transmission. OXA-48 genes are typically located on IncL/M plasmids, which are known for their efficient conjugation and stable inheritance, thus facilitating widespread dissemination within hospital environments [29].

The genotypic findings are corroborated by BLAST analysis, which confirmed high sequence identity with reference OXA-48 genes deposited in GenBank. This reinforces the specificity of the primers used and validates the PCR-based approach for rapid molecular diagnostics. The accuracy and speed of PCR underscore its superiority over conventional phenotypic methods, which may lack sensitivity or fail to differentiate between enzyme types. Molecular methods, therefore, are indispensable for effective surveillance and outbreak management [30].

From a clinical standpoint, the rising prevalence of OXA-48-producing K. pneumoniae poses significant challenges. These strains are often resistant not only to carbapenems but also to multiple other antibiotic classes, leaving limited therapeutic options such as colistin and tigecycline—both associated with high toxicity and limited efficacy [31]. The high resistance rates to meropenem (33.97%) and ertapenem (37.82%) observed in the present study mirror national and international trends, where carbapenem resistance in Enterobacteriaceae continues to escalate [32,33].

Moreover, the statistical significance found in resistance variation (ANOVA, p = 0.00065) and distribution patterns (Chi-square test) highlights that carbapenem resistance is not a random occurrence but reflects an evolving epidemiological trend within the healthcare ecosystem. Targeted antimicrobial stewardship interventions, combined with active molecular surveillance, are essential to curb this trend [34].

Infection control policies must prioritize early detection of OXA-48 carriers to prevent nosocomial transmission. Asymptomatic colonization is common and may act as a silent reservoir, particularly in ICU and long-term care patients [35]. Screening protocols, contact precautions, and strict antibiotic stewardship are crucial components of any effective containment strategy.

CONCLUSION

The study underscores the predominance of OXA-48 carbapenemase among K. pneumoniae isolates in the clinical setting, with CRKP-2 notably absent. This reflects the regional molecular epidemiology of carbapenem resistance and emphasizes the need for precise, gene-level diagnostics in clinical microbiology. The findings advocate for enhanced molecular monitoring and strategic policy action to manage the growing threat of antimicrobial resistance in resource-limited healthcare systems.

REFERENCES

1. Nordmann P, Naas T, Poirel L. Global spread of Carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis. 2011;17(10):1791–8.
2. World Health Organization. Global priority list of antibiotic-resistant bacteria. Geneva: WHO; 2017.
3. Logan LK, Weinstein RA. The epidemiology of Carbapenem-resistant Enterobacteriaceae: The impact and evolution of a global menace. J Infect Dis. 2017;215(suppl_1):S28–S36.
4. Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL. Carbapenemases in Klebsiellapneumoniae and other Enterobacteriaceae: An evolving crisis of global dimensions. ClinMicrobiol Rev. 2012;25(4):682–707.
5. Pitout JD, Nordmann P, Poirel L. Carbapenemase-producing Klebsiellapneumoniae, a key pathogen set for global nosocomial dominance. Antimicrob Agents Chemother. 2015;59(10):5873–84.
6. vanDuin D, Doi Y. The global epidemiology of carbapenemase-producing Enterobacteriaceae. Virulence. 2017;8(4):460–9.
7. Yigit H, Queenan AM, Anderson GJ, Domenech-Sanchez A, Biddle JW, Steward CD, et al. Novel carbapenem-hydrolyzing β-lactamase, KPC-1, from a carbapenem-resistant strain of Klebsiellapneumoniae. Antimicrob Agents Chemother. 2001;45(4):1151–61.
8. Munoz-Price LS, Poirel L, Bonomo RA, Schwaber MJ, Daikos GL, Cormican M, et al. Clinical epidemiology of the global expansion of Klebsiellapneumoniaecarbapenemases. Lancet Infect Dis. 2013;13(9):785–96.
9. Poirel L, Potron A, Nordmann P. OXA-48-like carbapenemases: The phantom menace. J AntimicrobChemother. 2012;67(7):1597–606.
10. Carrër A, Poirel L, Yilmaz M, Akan OA, Feriha C, Cuzon G, et al. Spread of OXA-48-encoding plasmid in Klebsiellapneumoniae and Escherichia coli in Istanbul, Turkey. Antimicrob Agents Chemother. 2010;54(8):3505–8.
11. CLSI. Performance standards for antimicrobial susceptibility testing. 30th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2020.
12. Doyle D, Peirano G, Lascols C, Lloyd T, Church DL, Pitout JD. Laboratory detection of Enterobacteriaceae that produce carbapenemases. J ClinMicrobiol. 2012;50(12):3877–80.
13. Queenan AM, Bush K. Carbapenemases: The versatile β-lactamases. ClinMicrobiol Rev. 2007;20(3):440–58.
14. Veeraraghavan B, Shankar C, Karunasree S, Kumari S, Ravi R, Ralph R. Carbapenem resistant Klebsiellapneumoniae isolated from bloodstream infection: Indian experience. Pathog Glob Health. 2017;111(5):240–6.
15. Codjoe FS, Donkor ES. Carbapenem resistance: A review. Med Sci (Basel). 2017;6(1):1.
16. Mathers AJ, Peirano G, Pitout JD. The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant Enterobacteriaceae. ClinMicrobiol Rev. 2015;28(3):565–91.
17. Potron A, Poirel L, Nordmann P. Emerging broad-spectrum resistance in Pseudomonas aeruginosa and Acinetobacterbaumannii: Mechanisms and epidemiology. Int J Antimicrob Agents. 2015;45(6):568–85.
18. Partridge SR, Kwong SM, Firth N, Jensen SO. Mobile genetic elements associated with antimicrobial resistance. ClinMicrobiol Rev. 2018;31(4):e00088–17.
19. Nordmann P, Poirel L. Epidemiology and diagnostics of carbapenem resistance in Gram-negative bacteria. Clin Infect Dis. 2019;69(Suppl_7):S521–8.
20. Köseoğlu O, Gür D, Unal S. The emergence and spread of OXA-48-producing Enterobacteriaceae in Turkey. J Chemother. 2016;28(5):327–31.
21. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo-β-lactamases: the quiet before the storm? ClinMicrobiol Rev. 2005;18(2):306–25.
22. Cuzon G, Naas T, Nordmann P. Functional characterization of Tn1999, a Tn3-based transposon involved in mobilization of the blaOXA-48 carbapenemase gene. Antimicrob Agents Chemother. 2011;55(12):5370–3.
23. Doyle D, Peirano G, Lascols C, Lloyd T, Church DL, Pitout JD. Laboratory detection of Enterobacteriaceae that produce carbapenemases. J ClinMicrobiol. 2012;50(12):3877–80.
24. Veeraraghavan B, Shankar C, Karunasree S, Kumari S, Ravi R, Ralph R. Carbapenem resistant Klebsiellapneumoniae isolated from bloodstream infection: Indian experience. Pathog Glob Health. 2017;111(5):240–6.
25. Zowawi HM, Sartor AL, Sidjabat HE, et al. Molecular epidemiology of carbapenem-resistant Enterobacteriaceae in the Gulf Cooperation Council States: Dominance of OXA-48 and NDM producers. Antimicrob Agents Chemother. 2014;58(6):3085–90.
26. Carrër A, Poirel L, Yilmaz M, Akan OA, Feriha C, Cuzon G, et al. Spread of OXA-48-encoding plasmid in Klebsiellapneumoniae and Escherichia coli in

Istanbul, Turkey. Antimicrob Agents Chemother. 2010;54(8):3505–8.
27. Munoz-Price LS, Poirel L, Bonomo RA, et al. Clinical epidemiology of the global expansion of Klebsiellapneumoniaecarbapenemases. Lancet Infect Dis. 2013;13(9):785–96.
28. vanDuin D, Doi Y. The global epidemiology of carbapenemase-producing Enterobacteriaceae. Virulence. 2017;8(4):460–9.
29. Potron A, Poirel L, Rondinaud E, Nordmann P. Intercontinental spread of OXA-48 β-lactamase–producing Enterobacteriaceae over a 6-year period, 2001 to 2007. Antimicrob Agents Chemother. 2011;55(9):4375–8.
30. Nordmann P, Gniadkowski M, Giske CG, et al. Identification and screening of carbapenemase-producing Enterobacteriaceae. ClinMicrobiol Infect. 2012;18(5):432–8.
31. Tamma PD, Goodman KE, Harris AD, et al. Comparing the outcomes of patients with carbapenem-resistant and carbapenem-susceptible Enterobacteriaceae bloodstream infections: A multicenter retrospective cohort study. Clin Infect Dis. 2017;64(3):257–64.
32. World Health Organization. Antimicrobial resistance global report on surveillance. Geneva: WHO; 2014.
33. European Centre for Disease Prevention and Control (ECDC). Surveillance of antimicrobial resistance in Europe – Annual report 2022. Stockholm: ECDC; 2023.
34. Falagas ME, Tansarli GS, Karageorgopoulos DE, Vardakas KZ. Deaths attributable to carbapenem-resistant Enterobacteriaceae infections. Emerg Infect Dis. 2014;20(7):1170–5.
35. Doi Y, Hazen TH, Westblade LF. Screening and detection of carbapenemase-producing Enterobacteriaceae in the hospital setting: what is the gold standard? ClinMicrobiol Infect. 2021;27(9):1303–10.